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Pusterla, N ; Pusterla, J B ; Braun, Ueli ; Lutz, Hans

Abstract: The purpose of this study was to examine the seasonal variations in seroprevalence to Ehrlichia phagocytophila in cattle pastured during the summer months in an area where tick-borne fever is endemic. The study was performed during a 1-year period from April 1996 to March 1997 and involved 34 cows, 22 pregnant heifers, and 14 calves. Blood samples, collected from all 70 cattle once a month, were used to determine serum immunoglobulin G titers by indirect immunofluorescence. In addition, blood smears were examined for Ehrlichia organisms, and PCR amplification was performed for the molecular detection of E. phagocytophila. Prior to the pasture period, the seroprevalence was 16%. Two weeks after the start of pasturing, it was 43%, after which it progressively increased and reached a maximum of 63% in September. The seroprevalence progressively decreased after the end of pasturing to a low of 23%. The variation in antibody titers was similar to that of seroprevalence. E. phagocytophila organisms were detected in blood smears of 7 animals and by nested PCR in 12. Only four cows, which were on the pastures of endemicity for the first time, had clinical signs of ehrlichiosis. This study demonstrated marked seasonal variations in seroprevalence and in serum titers of antibody to E. phagocytophila in cattle. The incidence of clinical signs of ehrlichiosis was increased in cattle grazing on the pastures of endemicity for the first time.

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Serological, Hematologic, and PCR Studies of Cattle in an Area of Switzerland in Which Tick-Borne Fever (Caused by *Ehrlichia phagocytophila*) Is Endemic

NICOLA PUSTERLA,* JEANNINE BERGER PUSTERLA, UELI BRAUN, AND HANS LUTZ

Department of Veterinary Internal Medicine, University of Zurich, CH-8057 Zurich, Switzerland

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The purpose of this study was to examine the seasonal variations in seroprevalence to *Ehrlichia phagocytophila* in cattle pastured during the summer months in an area where tick-borne fever is endemic. The study was performed during a 1-year period from April 1996 to March 1997 and involved 34 cows, 22 pregnant heifers, and 14 calves. Blood samples, collected from all 70 cattle once a month, were used to determine serum immunoglobulin G titers by indirect immunofluorescence. In addition, blood smears were examined for *Ehrlichia* organisms, and PCR amplification was performed for the molecular detection of *E. phagocytophila*. Prior to the pasture period, the seroprevalence was 16%. Two weeks after the start of pasturing, it was 43%, after which it progressively increased and reached a maximum of 63% in September. The seroprevalence progressively decreased after the end of pasturing to a low of 23%. The variation in antibody titers was similar to that of seroprevalence. *E. phagocytophila* organisms were detected in blood smears of 7 animals and by nested PCR in 12. Only four cows, which were on the pastures of endemicity for the first time, had clinical signs of ehrlichiosis. This study demonstrated marked seasonal variations in seroprevalence and in serum titers of antibody to *E. phagocytophila* in cattle. The incidence of clinical signs of ehrlichiosis was increased in cattle grazing on the pastures of endemicity for the first time.

Ehrlichia phagocytophila is the cause of tick-borne fever, a disease of cattle characterized by pyrexia, decreased milk production, respiratory symptoms, and abortion (3). This obligate intracellular agent is transmitted naturally by the tick *Ixodes ricinus*. The disease occurs in most European countries, where its distribution is usually localized. In Switzerland, tick-borne fever was first reported in the Bernese Oberland (6). In a recent serological study involving 2,557 clinically healthy cattle from the eastern and central regions of Switzerland, 32 natural foci of tick-borne fever were identified. These foci represented areas with optimal conditions for the vector (7). The seroprevalence was dependent on several factors, including seasonal occurrence of ticks and pasture management. Clinical cases peak during spring and autumn when ticks are most active. In alpine regions, clinical cases are common during the early and late pasture periods, when meadows in wooded areas are grazed. Clinical cases are rare during midseason, when cattle are on alpine pastures above the tree line (8).

The purpose of this study was to investigate seasonal variations of the seroprevalence to *E. phagocytophila* in a region endemic for this agent and to monitor the number of diseased cattle by use of serial hematologic examinations and nested PCR.

MATERIALS AND METHODS

Animals. This study was performed from April 1996 to March 1997 and involved 34 cows, 22 pregnant heifers, and 14 calves from two different farms in a region endemic for *E. phagocytophila* in central Switzerland. The cattle belonged to the Swiss Brown breed and were between 3 months and 12 years old at the start of the study. During the winter the animals were housed in stalls. From the middle of May until the end of September 1996, all 70 cattle were put on a 50-ha community pasture in a subalpine-to-alpine region, 1,250 to 1,700 m above sea level. This pasture was surrounded by wooded areas and contained brush and ferns. It was the first time that all of the calves, two of the heifers, and eight of the cows had been on the pastures of endemicity, whereas the remaining cattle had been there during two or more pasture periods. During the pasture period, the cattle were examined once a month for tick infestation, which was scored as mild (<10 ticks/animal), moderate (10 to 30 ticks/animal), or severe (>30 ticks/animal). The incidence of tick-borne fever was monitored by the herdpersons, who notified us when cattle with clinical signs of tick-borne fever were observed.

Serological and hematologic examinations. At the beginning of each month, blood was collected from all 70 cattle from a jugular vein into evacuated glass tubes with and without anticoagulant (Becton Dickinson Vacutainer; Aichele Medico AG). Serum samples were examined for immunoglobulin G (IgG) to *E. phagocytophila* by indirect immunofluorescence using *E. phagocytophila* as antigen (Swiss strain) and fluorescein isothiocyanate-conjugated rabbit anti-bovine IgG as conjugated antisera (RAB/FITC; Nordic Immunological Laboratories b.v., Tilburg, The Netherlands). The cutoff titer was set at ≥20 as described previously (7). Smears made from EDTA blood were stained with May-Grünwald Giemsa stain, and 500 leukocytes were examined under oil immersion for the presence of intracytoplasmic inclusion bodies characteristic of *E. phagocytophila*.

Nested PCR. Heparinized blood samples (10 ml) were collected from each animal. The procurement of a leukocyte pellet, the isolation of DNA, and the procedure of nested PCR were done as described previously (9). Products from nested PCR were resolved on 1.2% agarose gels, stained with ethidium bromide, and examined under UV illumination.

RESULTS

Prior to pasturing, 59 (84%) of the cattle had titers of <20 and 11 (16%) had titers of 20 or 40; the latter comprised six cows and five heifers, which had been on the pastures of endemicity for at least one pasture period. Two weeks after the start of pasturing, the seroprevalence was 43%. During the following months, there was a progressive increase in the seroprevalence, which reached a maximum of 63% at the end of the pasture period. After the pasture period, the seroprevalence decreased and in March of the following year was 23% (Table 1).

There was an increase in titers parallel to the increase in seroprevalence. Towards the end of the stable period, titers were generally as low as 40. Shortly after the start of the

* Corresponding author. Mailing address: Klinik fur Wiederkauer- und Pferdemedizin, Universitat Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland. Phone: (41) 1 635 83 51. Fax: (41) 1 635 89 06. E-mail: pusterla@vetmed.unizh.ch.

TABLE 1. Seroprevalence of 70 calves, heifers, and cows in an area endemic for tick-borne fever from April 1996 to March 1997

Mo	Seroprevalence[a] (%[b])	No. of seropositive[a] animals[c]		
		Calves	Heifers	Cows
April	19	0	6	7
May	16	0	5	6
June	43	5	10 (2)	15 (3)
July	54	7	13 (2)	18 (5)
August	59	8	14 (2)	19 (5)
September	63	9	14 (2)	21 (6)
October	61	9	13 (2)	21 (6)
November	60	8	13 (2)	21 (6)
December	46	6	8	18 (5)
January	34	5	6	13 (4)
February	27	5	5	9 (2)
March	23	4	4	8 (2)

[a] Titer, ≥20.
[b] Of the 70 animals.
[c] The number of animals pastured on the fields of endemicity for the first time is indicated in parentheses.

pasture period, the titers began to increase and reached a maximum in August and September. Thereafter, the titers decreased progressively and ranged from 20 to 640 in November, from 20 to 160 in January, and from 20 to 40 in March (Table 2). There were no differences in seroprevalence and titers between cattle that were pastured for the first time and those that had been on the pastures of endemicity before.

E. phagocytophila inclusion bodies were detected in blood smears of five heifers and two cows (Table 3). In six animals this occurred 2 weeks after the start of the pasture period, and in one it occurred 10 weeks after the start. All animals were asymptomatic at the time of blood collection. It was the second season for these heifers and the first season for these cows on the pastures of endemicity.

Nested PCR yielded products of 928 bp amplified from *E. phagocytophila* DNA in 12 cattle (Table 3). These included the seven cattle that had inclusion bodies and an additional heifer and four cows. The latter had been on the pastures of endemicity during at least one previous summer, and the positive PCR product occurred 2 weeks after the move to the pasture. Clinical signs were not observed in any of the cattle at the time of blood collection. The antibody titer in animals positive by blood smear and/or PCR was ≤20 and reached the highest level 30 to 60 days later. None of the 70 animals had detectable positive blood smears or PCR results prior to pasturing or during the following stall period.

Tick infestation was assessed as moderate in May and June, mild in July and August, and severe in September. Four cows had clinical signs typical of tick-borne fever (drop in milk yield and pyrexia) combined with *E. phagocytophila* inclusion bodies in circulating neutrophils and were treated with tetracycline. These cows were on the pastures of endemicity for the first time. Two cows developed clinical signs 1 day after the monthly blood collection, 2 and 10 weeks after moving to the pastures of endemicity, respectively. In these two cows, *E. phagocytophila* was identified by blood smear and PCR (Table 3). One of the cows became ill 4 weeks after being moved to the pastures of endemicity, and the other became ill 16 weeks after the move. At the time of the subsequent blood collection 2 weeks later, these two cows had negative blood smears and negative PCR results.

DISCUSSION

These investigations have demonstrated marked seasonal variations in seroprevalence and serum titers of IgG to *E. phagocytophila* in cattle. The marked increase in seroprevalence in June, merely 2 weeks after the move to the pastures of endemicity, indicates that numerous cattle had contact with *E. phagocytophila* during this short period. Other cattle were probably still in the incubation period. Previous studies have shown that seroconversion occurs 6 to 11 days after infection under natural as well as experimental conditions (9, 13). The progressive increase in seroprevalence in the months of July and August can be explained by the fact that these cattle, in contrast to other alpine farming practices, were not moved to higher, tick-free meadows. The peak in seroprevalence occurred in September, which can be explained by a seasonal increase in tick activity. In Switzerland, ticks are most active in spring and autumn, when temperatures and humidity are favorable. During the summer months, ticks move away from the higher parts of the vegetation to seek a more favorable microclimate near the ground (1). The progressive decrease in seroprevalence during the following stable period can be explained by the lack of antigenic stimulation and the natural decline in the antibody concentration over a period of approximately 7 months. A decline in titers of this magnitude corresponds to a half-life of bovine IgG of 17 to 22 days (11).

TABLE 2. Serum titers of IgG to *E. phagocytophila* in 70 calves, heifers, and cows in an area endemic for tick-borne fever from April 1996 to March 1997

Mo	No. of animals with the following titer[a]:							
	<20	20	40	80	160	320	640	1,280
April	57 (14, 16, 27)	8 (0, 4, 4)	4 (0, 1, 3)	1 (0, 1, 0)	0	0	0	0
May	59 (14, 17, 28)	9 (0, 4, 5)	2 (0, 1, 1)	0	0	0	0	0
June	40 (9, 12, 19)	3 (1, 1, 1)	9 (2, 2 [1], 5)	11 (2, 5 [1], 4 [1])	4 (0, 1, 3 [2])	3 (0, 1, 2)	0	0
July	32 (7, 9, 16)	2 (1, 0, 1)	11 (2, 5, 4)	10 (2, 4 [1], 4 [2])	9 (2, 3 [1], 4 [1])	1 (0, 0, 1)	5 (0, 1, 4 [2])	0
August	29 (6, 8, 15)	3 (0, 1, 2)	5 (2, 2, 1)	10 (1, 4 [1], 5)	11 (2, 4 [1], 5 [1])	5 (2, 1, 2 [1])	6 (1, 2, 3 [2])	1 (0, 0, 1 [1])
September	26 (5, 8, 13)	3 (1, 0,2)	5 (2, 1, 2)	12 (1, 5, 6 [1])	8 (1, 3 [1], 4 [1])	7 (2, 1, 4 [2])	8 (2, 3 [1], 3 [2])	1 (0, 1, 0)
October	27 (5, 9, 13)	5 (2, 2, 1)	11 (2, 2 [1], 7 [1])	9 (1, 4, 4)	9 (2, 2 [1], 5 [3])	7 (2, 2, 3 [1])	2 (0, 1, 1 [1])	0
November	28 (6, 9, 13)	15 (2, 8 [2], 5)	9 (1, 1, 7 [1])	8 (3, 1, 4 [3])	6 (2, 1, 3 [1])	3 (0, 2, 1 [1])	1 (0, 0, 1)	0
December	38 (8, 14, 16)	11 (1, 4, 6 [1])	10 (3, 1, 6 [1])	8 (2, 2, 4 [2])	2 (0, 0, 2 [1])	1 (0, 1, 0)	0	0
January	46 (9, 16, 21)	8 (3, 2, 3 [1])	12 (2, 2, 8 [2])	3 (0, 1, 2 [1])	1 (0, 1, 0)	0	0	0
February	51 (9, 17, 25)	14 (5, 3, 6 [1])	4 (0, 1, 3 [1])	0	1 (0, 1, 0)	0	0	0
March	54 (10, 18, 26)	12 (4, 3, 5 [2])	4 (0, 1, 3)	0	0	0	0	0

[a] Reciprocal of the serum dilution. Numbers of calves, heifers, and cows, in that order, are indicated in parentheses; numbers of animals pastured on the fields of endemicity for the first time are indicated in brackets.

TABLE 3. Hematologic, PCR, and antibody titer results for cattle infected with *E. phagocytophila*

Animal[a] (pasture period[b])	Blood smear[c]	PCR result[d]	Titer[e]	Highest titer (mo)
Heifer (2)	+	+	20	160 (August)
Heifer (2)	+	+	20	640 (August)
Heifer (2)	+	+	<20	160 (July)
Heifer (2)	+	+	20	80 (July)
Heifer (2)	+	+	<20	80 (July)
Cow[f] (1)	+	+	<20	640 (July)
Cow[f,g] (1)	+	+	<20	1,280 (August)
Heifer (2)	−	+	<20	640 (July)
Cow (2)	−	+	20	160 (July)
Cow (2)	−	+	<20	80 (August)
Cow (>2)	−	+	20	160 (July)
Cow (>2)	−	+	<20	160 (July)

[a] Blood samples were taken in June, unless indicated otherwise.
[b] Number of seasons on the pasture of endemicity.
[c] Detection of *E. phagocytophila* inclusion bodies.
[d] Detection of a 928-bp amplified product.
[e] Reciprocal of the serum dilution.
[f] Clinical signs were observed 1 day after blood collection.
[g] The sample was obtained in July.

Reports on seasonal variations in seroprevalence to *E. phagocytophila* in domestic animals are rare. A study of sheep in Scotland and the northern parts of England showed a peak in seroprevalence (31.4%) in the month of July (13).

We could clearly demonstrate that infection with *E. phagocytophila* occurred predominantly at the beginning of the pasture period. Although a single hematologic and nested-PCR examination per month does not permit a quantitative statement regarding disease incidence, we were able to draw several important conclusions from this study. As in a previous report (9), nested PCR proved more sensitive compared to examination of blood smears for inclusion bodies. Particularly in the early and late stages of the disease, the number of inclusions may be too small for detection by light microscopy. Furthermore, only 2 of the 12 animals with positive PCR had clinical signs of tick-borne fever. These two cows had not been on the pastures of endemicity before. In the other 10 cattle (6 heifers and 4 cows that had been on the pastures of endemicity previously), clinical signs of ehrlichiosis were either missed or not apparent. This suggests that the initial exposure to a pasture of endemicity increases the risk of clinical ehrlichiosis and that immunity acquired in the previous pasture season may be insufficient to prevent infection with *E. phagocytophila* but sufficient to prevent clinical signs. Interestingly, clinical signs of ehrlichiosis were not observed in calves, although they were on the pastures of endemicity for the first time. However, in contrast to lactating cows and pregnant heifers, calves are generally subjected to less intensive management and not monitored as regularly. Furthermore, in young cattle, ehrlichiosis has a milder course than in older cattle, provided that secondary infection does not occur (2, 4, 5, 10, 12).

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